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ORIGINAL ARTICLE

Simultaneous Analysis of 15 Synthetic Cannabinoids in Human Urine by Using Liquid-Chromatography Tandem-Mass Spectrometry

Mukaddes GÜRLER*, [MD]

* Hacettepe University Faculty of Medicine, Department of Medical Biochemistry and Forensic Medicine, Ankara, Turkey

Phone: +90 312 305 1652

e-mail: mukaddes.gurler@hacettepe.edu.tr

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ABSTRACT

Synthetic cannabinoids became popular since 2004 among mind-altering drugs, called as "legal highs", "designer drugs", or "herbal highs". They are sprayed on plants and used by smoking, vaporizing or inhalation. They act on the same receptors (CB1 and CB2) as delta-9-tetrahydrocannabinol (Δ-9-THC) and are much more potent on brain cells than THC. To overcome the legal obstacles new analogues of synthetic cannabinoids are produced continuously by changing their chemical formulas. Immunochemical methods for detection are difficult to develop due to the substantial structural variety and cross-reactivity of these substances. Therefore, independent confirmation methods are usually required such as gas- or liquid-chromatography-mass spectrometry techniques (GC-MS and LC-MS/MS respectively).

We developed a validated LC-MS/MS method for the analysis of 15 synthetic cannabinoids simultaneously in human urine and applied it to a set of routine clinical and forensic toxicology cases. We studied the linearity, limits of detection and quantification (LOD and LOQ respectively), accuracy of repeatability and reproducibility, recovery and carry-over as validation parameters. We assessed all results in acceptable ranges. Bias and RSD values were less than ± 15% and 15%, respectively. Calibration curve was linear (R2=0.999) in the range of 1-20 ng/mL. LOD and LOQ were found between 0.7 - 1.07 and 2.19 – 3.56 ng/mL, respectively.

Key Words: Synthetic cannabinoids, LC-MS/MS, method development, validation, urine analysis

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INTRODUCTION

In recent years, new psychoactive substances (NPS) called as "legal highs", "designer drugs", or "herbal highs" are increased overall the world. Synthetic cannabinoids were available on the market and became popular since 2004 among mind- altering drugs [1]. In general, synthetic cannabinoid containing drug is called as "Spice" in Europe, "K2" in the US, whereas "Bonzai" or "Jamaica" in Turkey [2]. These chemicals are mainly sprayed on plants and used by smoking, vaporizing or inhalation (e-cigarette) as herbal or liquid incense. Acting on the same receptors (CB1 and CB2) as Δ-9-THC (delta-9-tetrahydrocannabinol), they are called as cannabinoids and often marketed as safe "legal substances" alternative to THC. However, their effect on brain cells are much more potent, unpredictable and sometimes life-threatening than THC [3]. Owing to absorption through lungs immediately after smoking and redistribution to other organs in a short time, the effect of cannabinoids starts in a few minutes [4]. They are lipophilic substances and probably have high distribution volume. Therefore, they can be stored in lipid containing tissues after chronic administration. According to the World Drug Report 2015, synthetic cannabinoids continued to account for the majority of NPS in 2014 [5]. Some of these materials have been around for years but came back the market in changed types and sold especially through the internet. Due to the high potential for abuse and ability to cause serious health problems or death the authorities banned them. However, to overcome the legal obstacles the manufactures have been producing new

analogues of synthetic cannabinoids continuously by changing the chemical formulas [6]. Herbal products containing synthetic cannabinoids in a variety of amount and type have included Spice Gold, Spice Silver, Spice Diamond, Yucatan Fire, Sence, Chill X, Smoke, Genie, Algerian Blend and many others [5,7].

Because of the high potential to be addicted to these substances and to reply to the increasing request for synthetic cannabinoids analysis, especially from Emergency Departments, laboratories have developed various analytical methods [8].The improvement of selective immunoassay methods for detecting synthetic cannabinoids is difficult due to the substantial structural diversity of this group. Their quantitative analysis is restricted due to the availability of pure reference standard samples. Furthermore, as the ingredient of 'herbal mixtures' is both steadily varying and widening, present methods cannot be sufficient to detect the new substances. Immunochemical procedures are easy and fast to perform and offer satisfied results in most conditions, but their specificity is too low to separate and identify particular drugs in the samples. It is also well known that immunoassays can produce false positive (cross-reactivity) or false-negative (lack of desired sensitivity) results in some cases. Therefore, more selective and sensitive analytical techniques are usually required for confirmation. Gas chromatography-mass spectrometry (GC-MS) or gas chromatography-tandem mass spectrometry (GC-MS/MS) have been used for this purpose [9-14]. Despite GC-MS or GC-MS/MS provides required analytical performance for detecting a wide range of compounds, these analyses are usually time-consuming and result in sample loss in most cases because of derivatization step during sample preparation, last but not least the heat-unstable substances are degraded even in the injection port of the instrument. Recently LC-MS (liquid chromatography- mass spectrometry) or LC-MS-MS (liquid chromatography-tandem mass spectrometry) methods have been preferred assays with more sensitive, specific, fast and easy extraction method [15-20]. Biological specimen selection is a critical point in these analyses. Urine is accepted as a more useful sample for drug testing owing to the fact that sample collection is non-invasive than those of blood, particularly, when the person is not entitled to urge blood sampling or medical staff is not available. Parent synthetic cannabinoids (non-metabolized) can be easily quantified in serum or whole blood following exposure within hospitals, psychiatric and detoxification centers. However, in workplace drugs-of-abuse testing or in some forensic cases, serum may not be useful or adequate for routine screening and detecting the suspected drugs (because of their short half-life in blood), so it

screening and detecting the suspected drugs (because of their short half-life in blood), so it is essential to use urine as the preferred matrix [16,17,20]. Present study is about developing a validated LC/MS/MS method for the detection of 15 synthetic cannabinoids simultaneously in urine using a simple and quick sample preparation

MATERIALS and METHODS

Standards and chemicals

All certified drug and isotope-labeled internal standard solutions [JWH-073, JWH-073-N-(2-hydroxybutyl), JWH-081, JWH-122, JWH-200, JWH-250, UR-144, UR-144-N-(5-hydroxypentyl), UR-144-N-pentanoic acid, AM-2201, JWH-018, JWH-018-N-(5- hydroxypentyl), JWH-018-N-(5-pentanoic acid), RCS-4, XLR-11, and JWH-018-d11) were purchased from Lipomed (Swiss Health Care Company). Structures of all compounds included in the method are illustrated in Figure 1. All chemicals (methanol, acetonitrile, ammonium formate, formic acid) were of LC-MS gradient grade and were purchased from Sigma Aldrich (Missouri, USA). Ultra-pure water (18.1 MΩ) was produced by a Mes Mp Minipure water system (MPMINIPURE,Turkey).

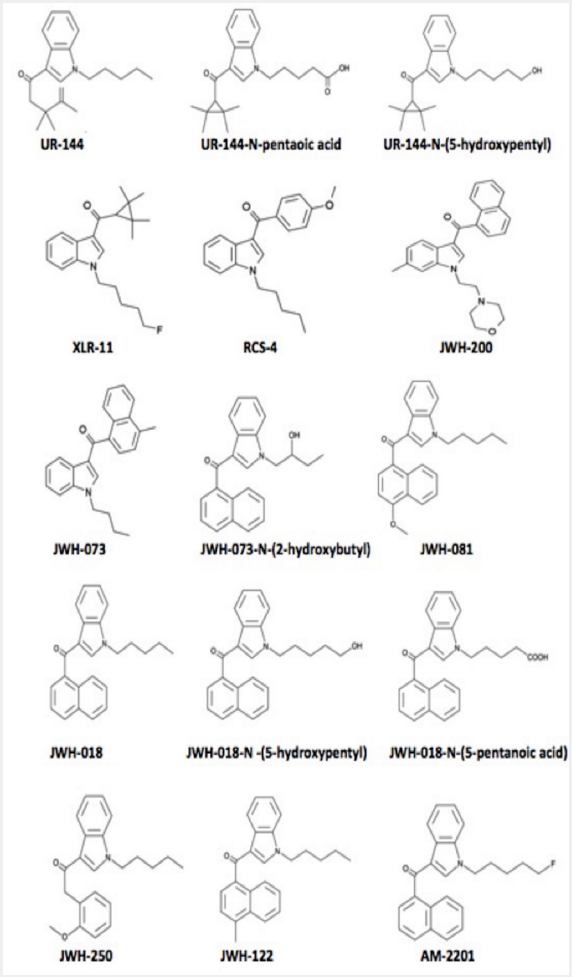

Figure 1. Structures of parent and metabolite compounds of synthetic cannabinoids included in the method

INSTRUMENTAL CONDITIONS

The LC part (Nexera XR, Shimadzu) comprised of two LC-20AD XR pumps, a DGU-20A3R in-line degasser, a SIL-20AC XR autosampler, and a CTO-10AS VP column oven. The MS/MS system used was Shimadzu 8030-Plus, with an ESI (electrospray ionization) source. Electrospray ionization was employed in positive mode (ESI+) for all analytes. The flow rates of nebulizing and drying gas were 1.5 L/min and 10 L/min, respectively. Data acquisition and quantitative analysis were performed using Lab Solutions Version 5.80 software (Shimadzu). Multiple reaction monitoring (MRM) method parameters were optimized by direct injection of standard solutions. The most abundant MRM transition was selected for quantification and the retention times (RTs) were determined for schedule time of all substances.

Preparation of Calibration Standard Solutions and Control Materials

Blank urine pool constituted from samples collected from healthy volunteers (n=5) was verified to be negative for analytes. The stock solutions (1µg/mL) of certified materials and internal standard (IS) were prepared in methanol and were stored at -20°C.Seven-point calibration curves were constructed daily by adding all standard stock solutions into blank urine at various concentrations (1.0, 2.5, 5, 7.5, 10, 15, and 20 ng/mL). Positive urine quality control (QC) samples (2.5, 7.5 and 20.0 ng/mL) were prepared daily and freshly by using the stock standard sources, separately from calibrators. A blank urine, as negative control, was included in every batch. Each calibrator and control sample was spiked with internal standard (100ng/mL JWH-018-d11).

SAMPLE PREPARATION

The samples were first mixed with acetonitrile (v/v=1/1), and then centrifuged for 5 min at 3500 g. The supernatant (200µL) was transferred to the auto sampler vial and fortified with internal standard (100ng/mL). The total volume was completed to 1mL with ultrapure water, before

LC-MS/MS injection.

CHROMATOGRAPHIC CONDITIONS

The chromatographic separation was performed using a Shim-Pack Column FCODS (150 mm x 2.0 mm, 3 µm, Shimadzu). The aqueous mobile phase (phase A) consisted of 10 mM ammonium formate in water, while the organic mobile phase (phase B) consisted of methanol. The column oven temperature was maintained at 40°C, and the flow rate was 0.4 mL/min. The initial gradient conditions (50% B) increased to 95% B over 10 min, held for 5 min, decreased to 5% and returned to initial conditions over 7 min, lasting a total run time of 22 min. The injection volume was 10 µL both for calibrators and samples.

VALIDATION

The method was validated for linearity, sensitivity (LOD: limit of detection and LOQ: limit of quantification), selectivity, inter and intraday-run and between laboratory staff (n=3) precision and accuracy, recovery and carry-over according to method validation guides [21,22]. The linearity was assessed by the analysis of calibration standard samples (1, 2.5, 5, 7.5, 10, 15, and 20 ng/mL) with three replicates at each level and drawing the calibration curve for each analyte. Linearity was acceptable if regression coefficient (R2) was equal or close to 0.999. Sensitivity was assessed establishing the limit of detection (LOD) and quantification (LOQ) for each analyte according to average mean value (x) of ten times repeated blank urine analysis and obtaining the standard deviations (SD). LOD and LOQ were calculated by the equation of x+3SD and x+10SD, respectively. A value near to the LOD was defined as the lowest concentration of the standard curve that could be measured with acceptable accuracy and imprecision. Acceptable accuracy (bias) and imprecision (RSD) was ± 20%, and below 20% respectively for the lowest calibrator.The selectivity or specificity is evaluated by analyzing blank and fortified blank matrix with reference standards in low and high concentrations and ensuring that they do not produce any interference for all analytes at the appropriate RTs.Blank urine samples were spiked with standard solutions to obtain 2.5 ng/mL (low), 7.5 ng/mL (mid), and 20 ng/mL (high) QC samples. Each spiked sample was prepared in three vials separately and injected for six times. Averages, standard deviations and biases were calculated to determine the precision and accuracy for repeatability and reproducibility at the three QC levels. Precision is expressed as RSD (CV%), and accuracy is expressed as mean % deviation (bias) from the nominal concentration of the QC (2.5, 7.5, 20 ng/mL). Imprecision was acceptable if RSD was below 15% and accuracy was acceptable if bias was between ± 15% at each concentration. Carry-over was assessed injecting blank sample immediately and repeatedly (with 3 replicates) after the highest calibration standard samples. Carryover was considered insignificant, if the calculated results of blank samples were under the LOD level of each analyte.

Analytical recovery was determined from ten replicates at one QC concentration (7.5 ng/mL) across the linear dynamic range of the assay by comparing the mean result for all analysis to the nominal concentration value (i.e. mean % of expected concentration). Recovery values between 80-120 %, was acceptable.

RESULTS

Table 1 shows the RTs, MRM conditions and collision energies for the analytes. There were similar fragmentation ions and closer RTs among the substances, which caused difficulties in separation and identification of chemicals. Although, we extracted the total ion chromatogram successfully according to their product ions and RTs (Figure 2). The linearity of the assay was established between the highest (20 ng/mL) and the lowest (1ng/mL) calibration standards and regression coefficients (R2) were about 0.999 for all analytes. LOD was calculated between 0.7 and 1.07, while LOQ was found around 2.19 – 3.56. The recovery was between 98.02 – 102.7% for spiked samples at 7.5 ng/mL concentration (Table 1).Precision is expressed as RSD (CV%) and accuracy as bias, the mean % deviation from the nominal concentration of the QC samples (2.5, 7.5, 20 ng/mL). Intraday (four days) accuracy and precision values were found in the range of 0.02 - 3.13 and 1.4 - 8.59 at the lowest level of QC; 0.01 – 3.32 and 0.45 – 4.34 at the mid-

level of QC; 0.04 – 2.84 and 0.56 – 3.49 at the highest level of QC respectively (Tables 2). Accuracy and precision values between three laboratory staffs were in the range of 0.01 – 3.13 and 1.45 – 4.21 at the low level, 0.04 – 0.82 and 0.44 – 1.61 at the mid-level, and –0.68 – 4.37 and 0.62 – 2.41 at the highest level of QC respectively (Tables 3).

Table 1. MRM conditions and recovery (%), LOD **(**ng/mL), LOQ (ng/mL) and linearity (regression coefficients= R^2) values of synthetic cannabinoids

Analyte	ESI +/-	RT (min)	Q1 (m/z)	Q3 (m/z)	CE (V)	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	R^2 (1-20 ng/mL)
JWH-018-N-pentanoicacid	+	9.194	372.2	155.10 127.15 244.20	-25 -48	98.02	0.87	2.89	0.9985
UR-144-N-pentanoicacid	+	9.739	342.4	125.15 55.20 57.20	-22 -45 -44	100.9	0.7	2.33	0.9994
JWH-018-N-5-OH-pentyl	+	10.157	358.3	230.20 155.10 127.10	-25 -22 -47	100.3	1.07	3,56	0.9994
JWH-073-N-2-OH-butyl	+	10.171	344.3	155.10 144.10 127.10	-25 -36 -45	98.93	0.9	2.99	0.9990
JWH-200	+	10.192	385.3	155.15 114.20 127.15	-24 -26 -48	101.2	1.03	3.44	0.9994
UR-144-N-5-OH-pentyl	+	10.612	328.3	125.20 55.20 57.15	-20 -41 -45	100.2	1.06	3.53	0.9974
AM-2201	+	10.87	360.3	155.10 127.10 232.15	-26 -48 -24	102.3	0.71	2.36	0.9974
RCS-4	+	11.23	322.2	135.10 77.15 107.10	-25 -55 -41	100.2	0.9	2.99	0.9989
JWH-250	+	11.227	336.3	121.20 91.10 130.10	-21 -44 -40	102.7	1.03	3.45	0.9993
XLR-11	+	11.224	330.4	125.15 55.20 232.15	-24 -41 -25	101.8	0.82	2.74	0.9991
JWH-073	+	11.279	328.2	155.10 127.15 200.15	-24 -43 -23	99.28	0.86	2.86	0.9999
JWH-018-D11	+	11.531	353.3	127.10 155.10 225.20	-46 -26 -25	IS	IS	IS	IS
JWH-018	+	11.561	342.3	155.10 127.10 214.20	-25 -48 -24	101.2	0.9	2,98	0.9997
JWH-081	+	11.687	372.3	185.15 157.15 214.20	-26 -42 -25	102.2	0.8	2.66	0.9997
UR-144	+	11.841	312.4	125.25 55.20 214.20	-23 -40 -24	101.2	0.7	2.32	0.9991
JWH-122	+	11.826	356.4	169.15 141.10 214.20	-25 -43	101.4	0.66	2.19	0.9991

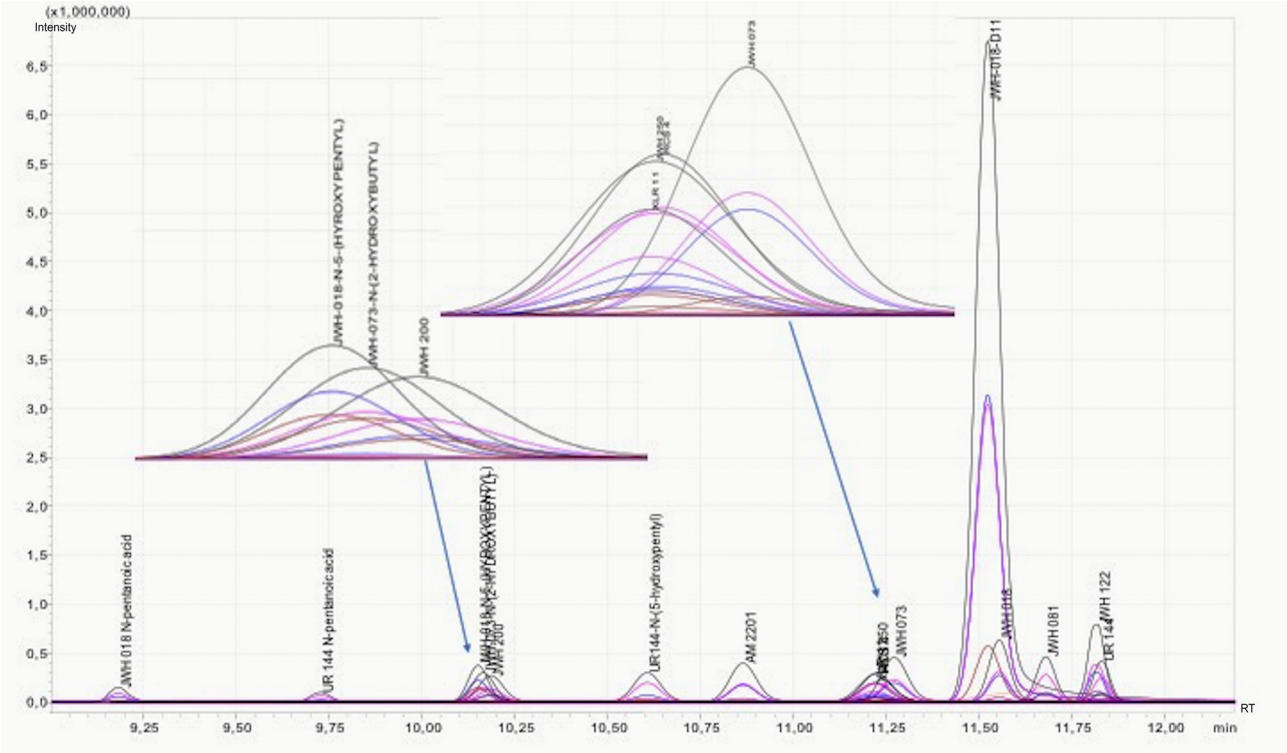

Figure 2. Total ion chromatogram of 15 synthetic cannabinoids according to their RTs (Table 1) and peak intensities (RT: retention time, expressed as minutes)

Table 2. Mean bias (accuracy) and RSD (precision) values for intra and interday-run (days 1-4) repeatability for three QC levels (2.5, 7.5, 20 ng/mL) of synthetic cannabinoids (results are expressed as % values)

	Level 1		Level 2		Level 3	
Analyte	Bias	RSD	Bias	RSD	Bias	RSD
JWH-073	1.36	1.69	0.75	2.19	0.63	1.32
JWH-073-N-2-OH-butyl	1.59	2.42	0.67	1.96	0.89	1.75
JWH-081	1.22	1.92	0.34	1.85	0.50	2.01
JWH-122	0.99	2.17	0.53	1.73	1.13	2.12
JWH-200	0.83	2.43	0.62	169	0.98	1.52
JWH-250	1.42	2.05	0.81	1.81	0.41	1.53
UR-144	2.16	1.62	0.65	1.84	0.81	2.08
UR-144-N-pentanoicacid	1.48	2.13	0.28	1.36	0.55	1.41
UR-144-N-5-OH-pentyl	1.25	1.99	0.99	1.13	1.24	1.00
AM-2201	1.96	1.97	1.20	2.03	1.28	1.41
JWH-018	1.49	240	1.06	1.86	0.65	2.10
JWH-018-N-5-OH-pentyl	2.03	2.37	0.50	1.82	0.43	1.75
JWH-018-N-pentanoicacid	1.55	4.08	0.75	1.11	0.77	0.91
RCS-4	1.38	2.47	0.52	1.75	1.40	1.29
XLR-11	1.33	2.08	0.64	2.23	0.99	1.44

Table 3. Mean bias (accuracy) and RSD (precision) values of repeatability of 3 laboratory staffs for three QC levels (2.5, 7.5, 20 ng/mL) of synthetic cannabinoids (results are expressed as % values)

	Level 1		Level 2		Level 3	
Analyte	Bias	RSD	Bias	RSD	Bias	RSD
JWH-073	0.93	1.99	0.50	1.23	0.92	0.98
JWH-073-N-2-OH-butyl	1.52	2.07	0.17	0.75	1.68	1.20
JWH-081	1.02	2.26	0.28	0.77	0.76	1.64
JWH-122	0.46	1.57	0.14	0.74	0.87	1.59
JWH-200	0.78	1.90	0.30	0.69	0.10	1.22
JWH-250	0.23	2.04	0.36	1.16	0.57	1.26
UR-144	0.61	2.45	0.32	0.53	1.08	2.16
UR-144-N-pentanoicacid	1.10	2.48	0.26	0.67	0.85	0.82
UR-144-N-5-OH-pentyl	1.46	1.76	0.22	0.55	3.18	1.31
AM-2201	1,69	2.47	0.38	1.14	1.34	1.26
JWH-018	1,50	2.39	0.29	0.96	0.73	1.49
JWH-018-N-5-OH-pentyl	1,65	3.21	0.15	0.78	1.46	1.39
JWH-018-N-pentanoicacid	1,53	1.82	0.35	0.94	1.28	1.22
RCS-4	0,26	2.31	0.28	0.67	2.08	1.20
XLR-11	0,59	2.39	0.35	1.04	1.30	1.58

Reproducibility was evaluated between the first and seventh day analysis at the three concentrations of QC samples. Bias and RSD values were found in the range of 0.02 – 6.36 and 1.08 – 3.62 at the low level, 0.07 – 3.31 and 1.8 – 4.75 at the mid-level, and 0.01 – 2.45 and 0.65 – 3.27 at the highest level of QC samples respectively (Tables 4).

Table 4. Mean bias and RSD values of reproducibility for three QC levels (2.5, 7.5, 20 ng/mL) of synthetic cannabinoids in the first and seventh day (results are expressed as % values)

	Level 1		Level 2		Level 3	
Analyte	Bias	RSD	Bias	RSD	Bias	RSD
JWH-073	0.60	2.06	0.44	3.61	1.39	1.41
JWH-073-N-2-OH-butyl	0.84	2.64	2.03	2.43	0.54	1.38
JWH-081	1.23	1.98	0.76	2.34	0.57	2.06
JWH-122	1.42	1.75	1.75	2.96	1.37	2.26
JWH-200	0.75	1.63	1.60	2.57	0.68	1.84
JWH-250	0.52	2.23	1.55	2.09	0.90	1.24
UR-144	0.95	2.07	0.53	3.67	0.81	2.98
UR-144-N-pentanoicacid	0.95	2.50	1.34	2.65	0.61	1.38
UR-144-N-5-OH-pentyl	1.05	1.87	0.69	2.49	0.15	1.21
AM-2201	1.76	2.39	0.09	2.22	0.68	1.90
JWH-018	1.17	2.24	0.92	3.39	1.03	2.28
JWH-018-N-5-OH-pentyl	2.25	2.43	1.00	3.38	0.81	1.69
JWH-018-N-pentanoicacid	3.67	3.06	0.31	2.57	0.22	1.43
RCS-4	0.39	2.45	0.58	2.93	0.08	0.79
XLR-11	0.75	2.21	0.28	2.98	1.95	1.44

samples injected after high concentrated samples (20 and 25 ng/mL). No peaks were observed at the appropriate RTs corresponding synthetic cannabinoids.

Evaluation of Routine Urine Samples

The method was applied to routine urine samples collected from the patients mainly submitted to pediatric emergency department and requested for drug of abuse tests. The ethical approval was obtained from the Ethical Committee of Clinical Research in June 2015. For detecting synthetic cannabinoids, urine specimens (n=25) were analyzed after hydrolysis as described before. But we also analyzed the same samples after enzymatic hydrolysis (because there was no positive results). For enzymatic hydrolysis, methanol and beta-glucuronidase (pH=5.5) were mixed with urine samples and incubated at 50oC for 2 hours and cooled under tap water. After applying the above described sample preparation procedure all samples were injected to LC-MS/MS with an injection volume of 10 µL.

DISCUSSION and CONCLUSION

The high popularity and availability of synthetic cannabinoids in Turkey has currently became a major public problem [23]. Therefore, the need for the administration of regulating control programs, and the need for the improving of analytical methods that can detect and quantify the synthetic cannabinoids in biological matrices, has increased. In this regard, our laboratory has been authorized as the first "confirmation laboratory" in drug of abuse analysis by the Ministry of Health in Turkey. The aim of this study was to develop an in-house validated method with an easy and quick sample preparation procedure for the quantitation of a range of synthetic cannabinoids in urine samples by using LC-MS/

Carry-over was assessed examining blank urine

MS. For this purpose, linearity, sensitivity (LOD and LOQ), selectivity, precision, accuracy, recovery and carry-over validation parameters were evaluated. The results of these parameters were found in acceptable ranges (RSD < 15% and bias ± 15%, respectively). We calculated the RSDs below 10% and accuracies (bias) in the range of ± 5% for all parameters. Knittel et al., validated quantitative methods for identifying synthetic cannabinoids in blood and urine [24]. They performed urine analysis by using Shimadzu MPX series liquid chromatograph coupled with an AB SCIEX 3,200 QTRAP LC–MS-MS. Their urine method's linear range, LOD, and LOQ were 0.1-10 ng/mL, 0.01-0.5 ng/mL, and 0.10 ng/mL respectively. The precisions and accuracies for intra and inter-day analysis were less than 12%, and 15% respectively, which are higher values than our findings. They calculated recoveries between 81.2-107.1%, which are less than our values (between 98-102%). Jang et al., established a validated method for the determination of 37 metabolites from 17 synthetic cannabinoids in urine by using LC-MS/MS (QTRAP) [25]. The LOD, linearity, precision and accuracies were 0.1-1.0 ng/mL, 0.25-100 ng/mL, 1.4-12% and -7.2-7.2%, respectively. Considering the real sample results, low concentrations of synthetic cannabinoids or their metabolites in urine have been reported [15-17]. The percentage of positive results among samples collected from emergence department, without a specific history of synthetic cannabinoid intake, corresponded to 2.5% with the concentration range of 0.05-3.9 ng/mL [15]. In urine samples from individuals who smoked 0.15 g herbal mixture, known to contain JWH-073 and JWH-018, the maximum concentration of synthetic cannabinoid metabolites was 10 pg/mL [17]. We analyzed the urine samples from drug suspected patients (no specific information of synthetic cannabinoid intake) admitted to pediatric emergency service. Our results were below the LOQ levels of all compounds. The patients may have not consumed any substance, or used another drug that contain different or new types of compounds which are not available in the method. It was not likely to obtain a full detection and quantification of all available/ marketed synthetic cannabinoids, for the reason that the proper analytical standards for all types of synthetic cannabinoids were not available. Consequently, despite efforts by developing new methods for detecting synthetic drugs and controlling with legal regulations, new types of drugs with similar chemical structure and metabolic fate have continually emerged on the market, especially the internet, to circumvent the regulations, and its abuse spreads among young children, that cannot be mainly detected with current methods. For this reason, we have to use more sensitive, selective and novel analytical methods with advanced techniques for the determination and identifying new synthetic drug compounds.

LIMITATIONS

Regarding to the chromatographic analysis of synthetic cannabinoids there are some analytical limitations and challenges. Co-eluting compounds and similar or overlapped mass spectra are common due to their structural similarities and isomeric forms. Therefore, these substances produce a complex chromatogram containing a substantial number of peaks that cause problems in identification. Reference material for confirmation positive results was difficult to obtain or did not exist. The cost of standard materials was too high to purchase all available standards. Libraries of reference mass spectra and RTs were not commercially available for screening all available synthetic cannabinoids by using LC-MS/MS. On the other hand, laboratory staff, available to analytical forensic method development and validation was a limitation during our initial studies and termination the all analytical processes took a while, leading new substances to emerge that we may not able to detect or catch them in urine samples with the current method.

CONCLUSION

In conclusion, we developed an LC-MS/MS method and validated for the confirmation and quantification of 15 synthetic cannabinoids simultaneously in urine samples, which is now being routinely applied together with those other validated methods for abused drugs in our forensic toxicology laboratory. Regarding to our preliminary results, there is a need to perform and

improve new analytical methods for the novel synthetic cannabinoids as much as possible, and apply it in clinical laboratories especially in cities where these substances are seized mostly.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

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